iGEM 2016 – SDU

Title: Competent cell - freeze-stock **SOP number:** SOP0022_v02 **Version number:** 02	**Date issued:** 2016.05.18 **Review date:** 2016.07.22 **Written by:** Brian Kenn Baltzar

1. Purpose

To make competent E. coli cells and to freeze them at -80°C

2. Area of application

This procedure is valid for all *E. coli* strains

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Freezer (-80 °C)	Laboratory (class 1) – V18-404b-0 Laboratory (class 2) – V15-501b-2		-75 °C - -80 °C
Pipette p1000			
15 ml tube rack	Hallway storage (1. Floor)		
Vortex			
Ice	Laboratory (class 1) – V18-404b-0		

4. Materials and reagents – their shelf life and risk labelling

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Glycerol	50 %	AppliChem	Anne Mette, RT	
Blue pipette tips		Contact Lab-manager	Micro storage	
Freezing tubes		Contact Lab-manager	Micro storage	
$CaCl_2$	0.1M		Chem room	
$MgCl_2$	0.1M		Chem room	
FalconTube		Contact Lab-manager	Micro storage	
liquid nitrogen				

5. QC – Quality Control

After completion of the SOP make a Ca^{++} transformation using a positive verified plasmid to check competent cells.

6. List of other SOPs relevant to this SOP

JMJ_SOP0001 ON culture of E.coli
iGEM 2016 SOP0023 - Ca++ Transformation

7. Environmental conditions required

8. Procedure

8.1 Incubate one colony from LB plate into 5 mL LB liquid medium. Shake at 37ºC overnight.
8.2 Incubate 400µL ON into 40 mL LB medium (in a 100 mL flask). Shake at 37 °C to $OD_{600}\sim$ 0.25-0.3(usually it takes about 2.5 hours).
8.3 Transfer the culture to a falcon tube
8.4 Chill the culture on ice for 5-20 min.
8.5 make sure the 0.1M $CaCl_2$ solution and 0.1M $MgCl_2$ are on ice
8.6 Centrifuge the cells for 10 min at 4ºC and 3500 G.
8.7 Discard the medium and resuspend the cell pellet in 20 mL cold 0.1M $MgCl_2$.
8.8 Centrifuge for 10 min at 4ºC and 3500 G.
8.9 Discard the medium and resuspend the cell pellet in 4 mL cold 0.1M $CaCl_2$.
8.10 Incubate the cells for 1-2 hours on ice.
8.11 Make 1 transformation using the SOP iGEM 2016 SOP0023 - Ca^{++} Transformation with a positive verified plasmid - to check for effect.
8.12 Transfer 500µL cells to freeze-tubes with 125µL 50% glycerol
8.13 Fast chill with liquid nitrogen
8.14 Label and put in -80ºC freezer

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks

ON Culture	Liquid bacterial waste
Once use plastic	GMO yellow waste

10. Time consumption

- Total-time 6-7h. + 16h for ON
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.05.18 / BKB	01	The SOP has been written
16.05.19/ MGJ	01	The SOP has been approved
16.07.22/BKB	02	Step. 8.11 was added - Make a transformation for control

12. Appendixes

12.1. When making transformation use SOP; iGEM 2016 SOP0023 - Ca++ Transformation